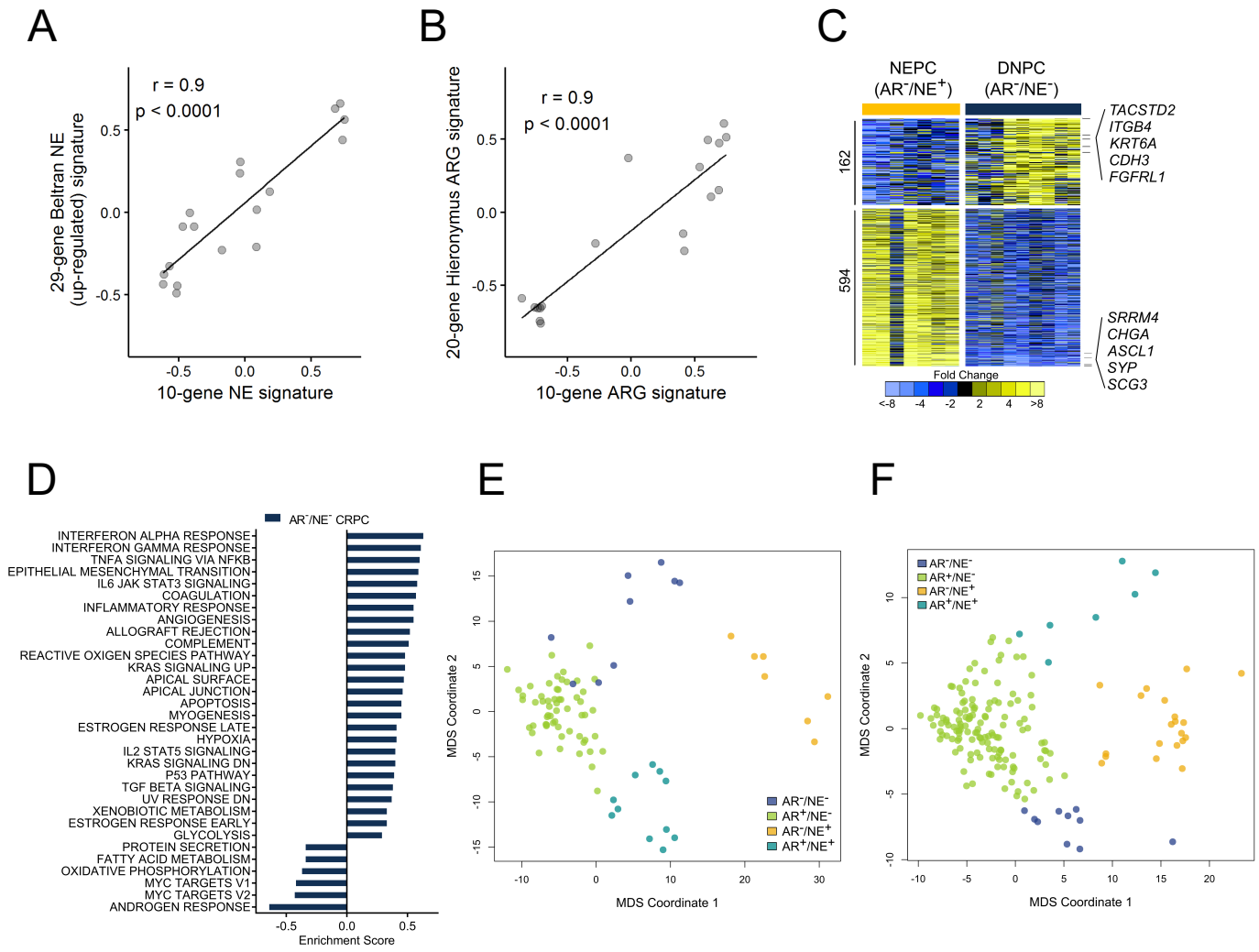


Figure S1. Related to Figure 1.

A. Correlation of 10-gene NE pathway activity and a published 29-gene NE up-regulated activity score (Beltran, et al. 2016) assessed across prostate cancer cell lines and LuCaP xenografts by RNAseq (see Figure S5). Pearson's correlation coefficient and p value are indicated on each plot.

B. Correlation of 10-gene AR pathway activity and a published 20-gene AR activity score (Hieronymus, et al., 2006) assessed across prostate cancer cell lines and LuCaP xenografts by RNAseq. Pearson's correlation coefficient and p value are shown.

C. Differentially expressed genes in AR-negative/neuroendocrine-positive (AR^-/NE^+) compared with AR-negative/neuroendocrine-negative (AR^-/NE^-) prostate cancer (5-fold difference; q value < 0.0001). Transcript abundance was determined by RNA sequencing and analyzed for differential expression using the Bioconductor edgeR software. (AR^-/NE^+ n = 7 tumors from 5 men; AR^-/NE^- n = 9 tumors from 5 men). Selected genes enriched in each phenotype are shown.

D. Gene Set Enrichment Analysis of genes differentially expressed between ARPC and DNPC.

E, F. MDS (multidimensional scaling) plots of CRPC expression profiles using expression of the AR, and a panel of 10 genes regulated by the AR in prostate cancer and a panel of 10 genes associated with neuroendocrine prostate cancer. E. RNA sequencing data from 85 CRPC tumors. F. Microarray expression data from 171 CRPC tumors.

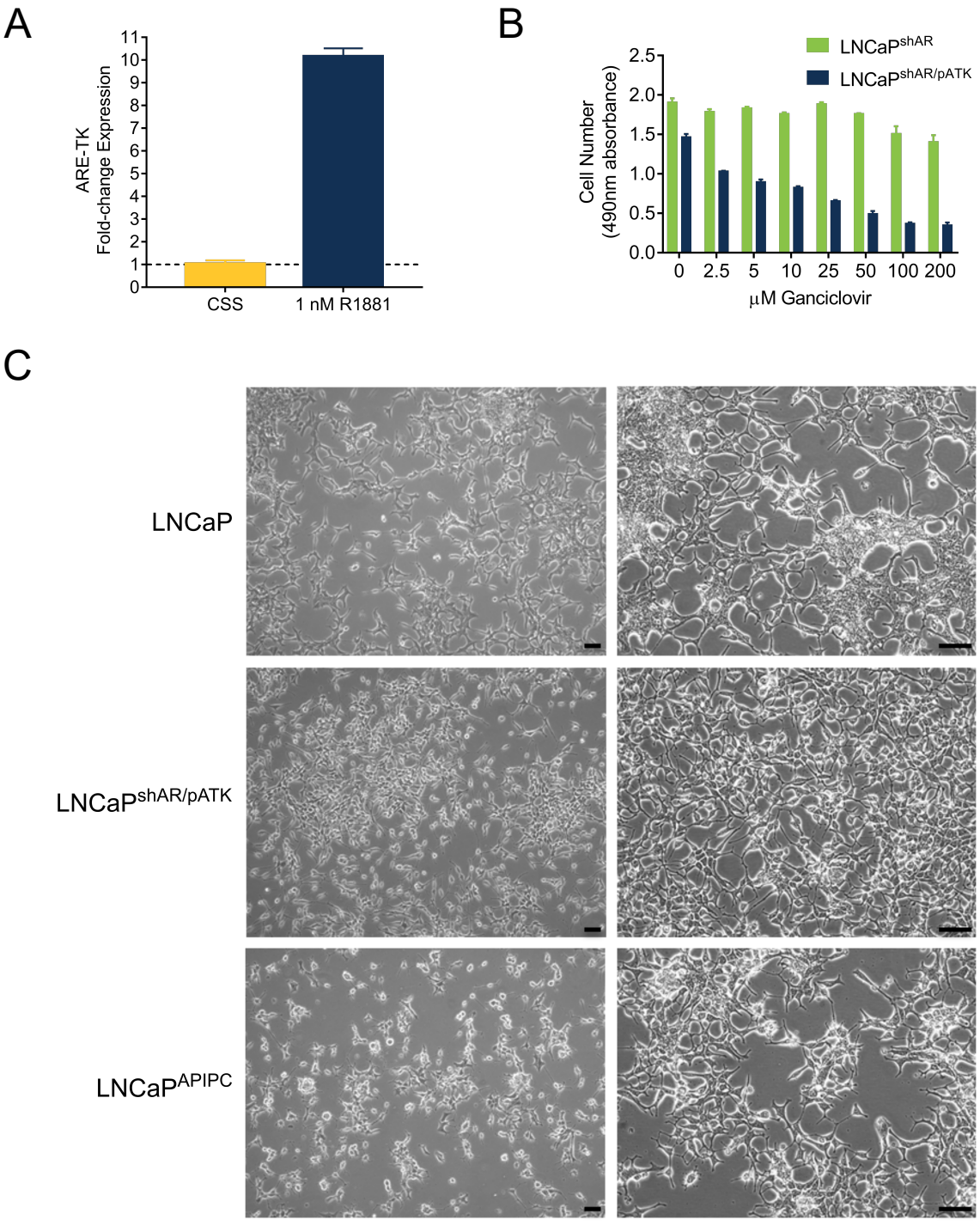
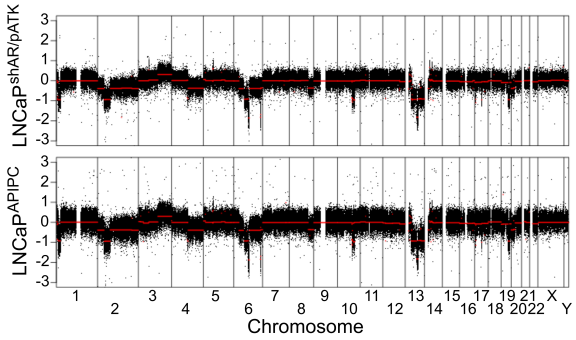
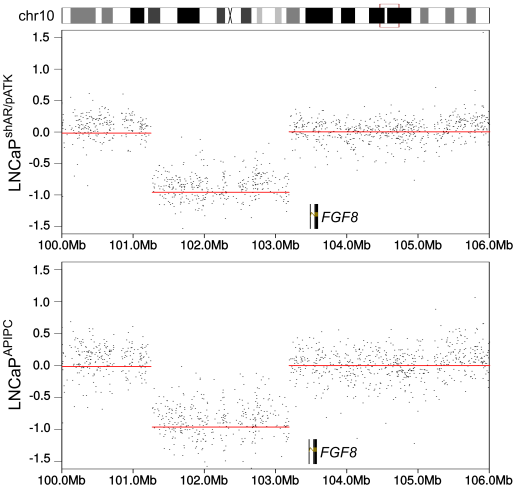


Figure S2. Related to Figure 2. Generation of LNCaP-APIPC, a model of DNPC.
A. HSV-TK expression in LNCaP$^{shAR/pATK}$ following exposure to the synthetic androgen R1881.
B. Graph of numbers of LNCaP$^{shAR/pATK}$ cells, and LNCaP^{shAR} cells without the AR-regulated thymidine kinase construct, grown in androgen-replete media with or without gancyclovir.
C. Representative images of LNCaP, LNCaP$^{shAR/pATK}$ and LNCaP^{APIPC} cells. Scale bars, 10 μm.
For A and B: Data are represented as mean ± SEM (n = 3 replicates per data point).

A



B



C

Sample	Chromosome	Gain/Loss	Start	End	Length (bp)	Genes in Region	Expression Δ
LNCaP^{APIPC}	Chr3	Gain	13,728,565	13,919,292	190,727	*LINC00620*, *WNT7A*	*, *
LNCaP^{APIPC}	Chr5	Gain	162,925,243	163,195,517	270,274	*MAT2B*	1.8
LNCaP^{APIPC}	Chr10	Gain	57,603,509	57,620,170	16,661	*LRP1*, *NXPH4*	-2.4, -1.6
LNCaP$^{shAR/pATK}$	Chr7	Loss	127,957,716	128,037,618	79,902	*RBM28*, *PRRT4*	1.4, *
LNCaP$^{shAR/pATK}$	Chr6	Gain	30,698,013	30,740,510	42,497	*FLOT1*, *IER3*	1.2, 1.3
LNCaP$^{shAR/pATK}$	Chr14	Gain	37,700,664	37,738,962	38,298	*MIPOL1*	-1.2
LNCaP$^{shAR/pATK}$	Chr18	Gain	34,523,407	34,648,291	124,884	*KIAA1328*	-1.8

Figure S3. Related to Figure 3. Analysis of LNCaP-APIPC, a model of DNPC.
A. Genome copy number alterations between LNCaP$^{shAR/pATK}$ and LNCaP^{APIPC} cells. Comparative genomic hybridization of LNCaP$^{shAR/pATK}$ and LNCaP^{APIPC} DNA against a normal reference standard was performed and copy gains or losses identified in one line but not in the other are shown in panel C.
B. Genomic region of *FGF8* on chromosome 10. Log^2 ratios of individual array probes are plotted, and copy number segments are indicated as red lines. No difference in the *FGF8* locus was observed.
C. Annotated genes found in each region altered between LNCaP$^{shAR/pATK}$ and LNCaP^{APIPC} are shown. Expression differences for genes in regions of loss or gain between the two lines were determined by RNA-seq quantitation of transcript abundance. Asterisk (*) indicate expression level below detection threshold (counts per million (CPM) mapped reads < 1.)

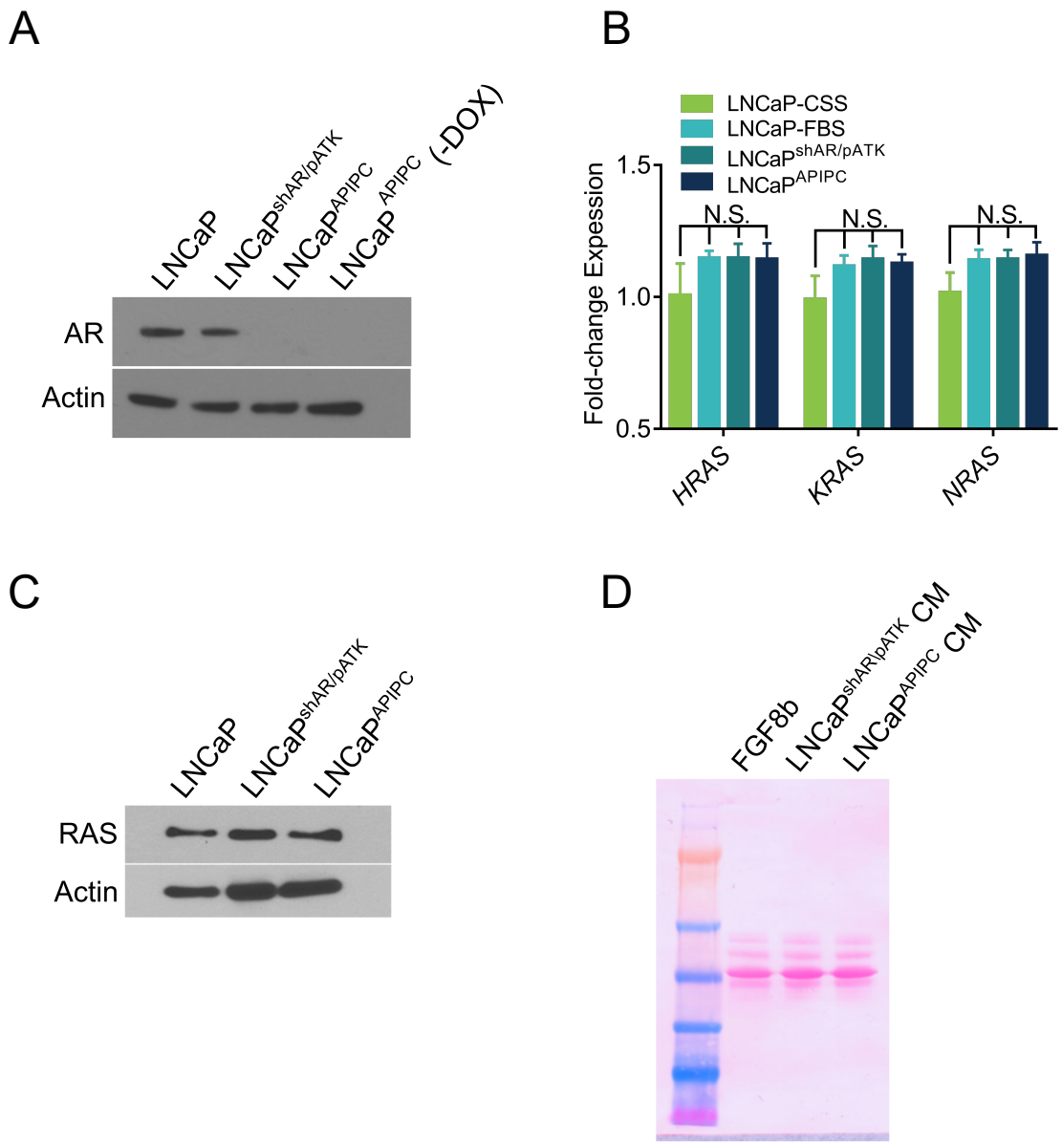


Figure S4. Related to Figure 3.

A. LNCaP^{APIPC} cells were grown for nine months without the addition of doxycycline to the media. Cells were then assayed by immunoblot for AR protein expression and compared with parental LNCaP and LNCaP^{APIPC} growth with doxycyline.

B. Transcript levels of RAS mRNAs quantitated by qRT-PCR in LNCaP, LNCaP$^{shAR/pATK}$ and LNCaP^{APIPC} cells. Transcript levels in LNCaP in CSS are set to a value of 1. Significance was determined using Student's T-test and data are represented as mean ± SEM (n = 3 replicates per data point).

C. Immuno-blot for RAS protein.

D. Ponceau stain as a loading control for the analysis of FGF8 in conditioned medium.

See also Figure 3J.

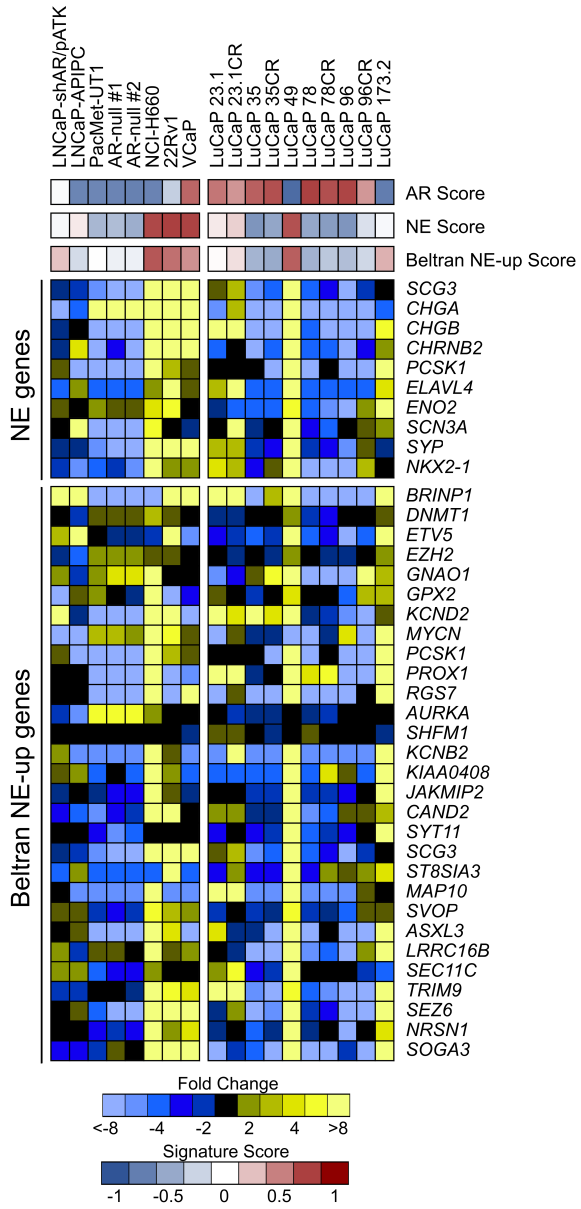


Figure S5. Related to Figure 5. Analysis of AR and NE expression signatures in prostate cancer cell lines and patient derived xenografts.
A 71-gene NE signature was divided into NE-up and NE-down subsets according to the direction of expression in NE samples (Beltran, et al. 2016). The expression of these genes were assessed across cell lines and LuCaP xenograft models and reduced to a single per-tumor activity score. These are shown in comparison with the 10-gene NE signature used to classify samples in this study. The left panel are prostate cancer cell lines and the right panel are prostate cancer patient derived xenografts (PDX). CR is castration resistant. NCI-H660 and LuCaP49 are classified as NE cancers based on immunohistochemistry for SYP expression. The remaining cell lines and PDX models are ARPC or DNPC.

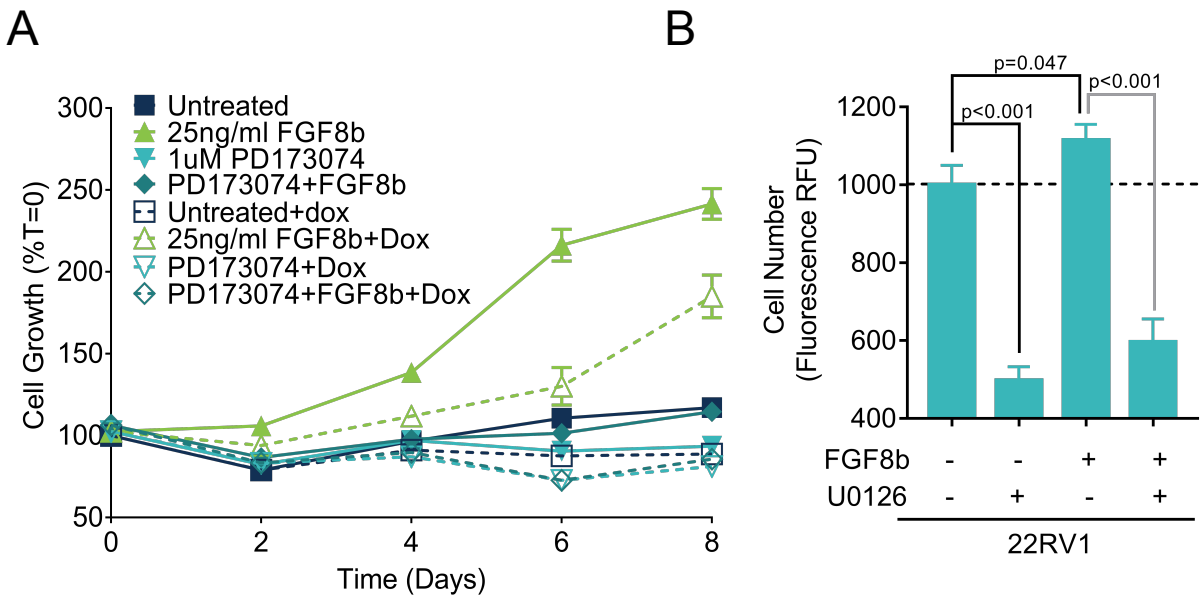


Figure S6. Related to Figure 6. Assessment of prostate cancer cell growth following AR pathway suppression and FGF8 exposure.

A. LNCaPshAR/pATK cells were cultured in androgen-depleted media and AR expression was suppressed by pre-treatment with 1 µg/ml doxycycline for 72 hours. Cell growth was also measured following the addition of exogenous FGF8b in the absence of AR ligand and AR suppression in LNCaPshAR/pATK by Dox (58% increase in growth compared to untreated LNCaPshAR/pATK, p = 0.003), and with the FGFR antagonist PD173074. Data points are mean ± SEM with n = 5 replicates per data point.

B. Measurement of 22RV1 cell number following exposure to FGF8b and the MAPK inhibitor U0126. Cell numbers were measured 3 days after treatment. Significance was determined using Student's T-test and data are represented as mean ± SEM with n = 3 replicates per data point. Dashed line is the cell number of control treatments for comparison with the treated cells.

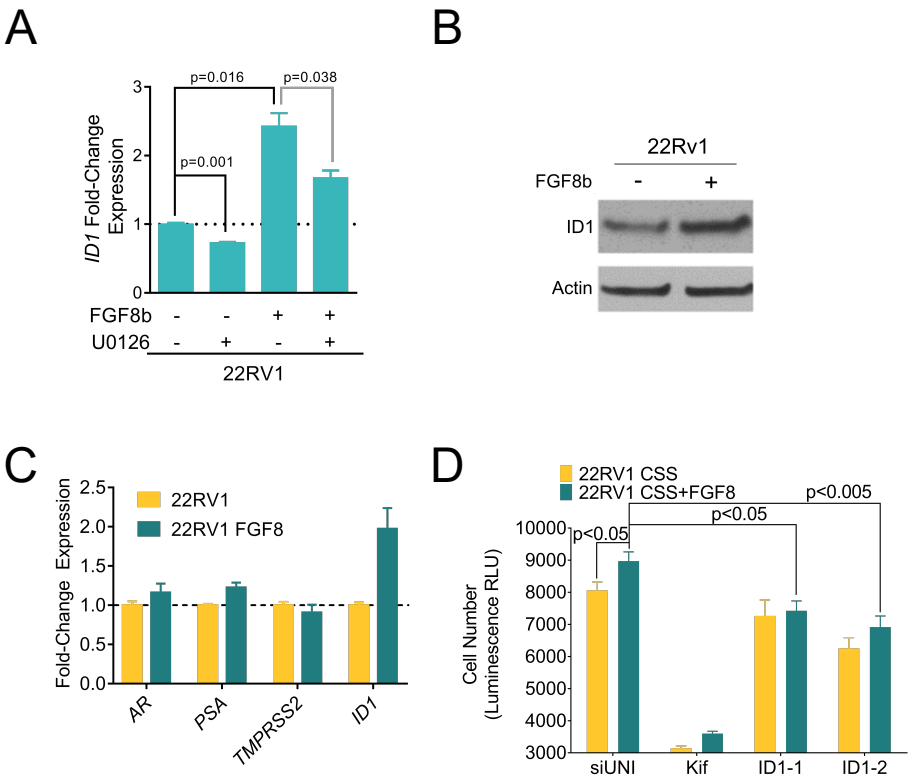


Figure S7. Related to Figure 7. Assessment of the growth of 22RV1 prostate cancer cells and ID1 expression following exposure to FGF8.

A. Measurement of ID1 transcript levels by qRT-PCR in 22RV1 cells following exposure to FGF8b and the MAPK inhibitor U0126.

B. Immunoblot of ID1 protein in 22RV1 cells following exposure to FGF8b.

C. Measurement of AR-regulated genes and ID1 by qRT-PCR following exposure to FGF8b.

D. Measurement of 22RV1 cell numbers in charcoal-stripped androgen depleted medium (CSS) with or without knockdown of ID1 by siRNA and with or without exposure to FGF8b. siUNI is a siRNA universal control. Kif is a cell death control using siRNAs targeting Kif11. All qRT-PCR experiments were repeated in triplicate.

For A, C, D: Significance was determined using Student's T-test and data are represented as mean ± SEM (n = 3 replicates per data point).

Table S1, related to STAR Methods. qRT-PCR Primers.

Gene	Forward Primer	Reverse Primer
AR	5'-ATCCTCATATGGCCCAGTGTC-3'	5'-GCTCTCTAAACTTCCCGTGGC-3'
HSV-TK1	5’-CACGTTATTTACCCTGTTTCGGGC-3’	5’-AGGATAAAGACGTGCATGGAACGG-3’
ID1	5’-AGGTAAACGTGCTGCTCTACG-3’	5’-TGTAGTCGATGACGTGCTGGA-3’
PSA	5’-GCATGGGATGGGGATGAAGTAAG-3’	5’-CATCAAATCTGAGGGTTGTCTGGA-3’
RPL13a	5’-CCTGGAGGAGAAGAGGAAAGA-3’	5’-TTGAGGACCTCTGTGTATTTG-3’
FGF8a/g	5'-CCAAGCCCAGCATGTGAGGGA -3'	5'-TCGGACTCGAACTCTGCTTCCAAA-3'
FGF8b	5'CTCCAAGCCCAGGTAACTGTT-3'	5'-TCGGACTCGAACTCTGCTTCCAAA-3'
FGF8e/f	5'-CTCGCTTCCCTGTTCCGGGCT-3'	5'-TCGGACTCGAACTCTGCTTCCAAA-3'
FGF9	5’-GGTTTCACAAACAGATGGTTA-3’	5’-ACAGATGGGAAGGTTGTTCAG-3’
FKBP5	5’-CGCAGGATATACGCCAACAT-3’	5’-GAAGTCTTCTTGCCCATTGC-3’
NKX3.1	5’-ACTAATGAGGTACGCTGAGGC-3’	5’-TGGCCAACTTTCTATTAACTTATG-3’
SOX2	5’-TTGCTGCCTCTTTAAGACTAGGA-3’	5’-CTGGGGCTCAAACTTCTCTC-3’
NSE	5’-AGGTGCAGAGGTCTACCATAC-3’	5’-AGCTCCAAGGCTTCACTGTTC-3’
CHGA	5’-CGCTGTCCTGGCTCTTCTG-3’	5’-TCACCTCGGTATCCCCTTTATTC-3’
SYP	5’-TTAGTTGGGGACTACTCCTCG-3’	5’-GGCCCTTTGTTATTCTCTCGGTA-3’
HRAS	5’-AAGCAAGGAAGGAAGGAAGG-3’	5’-GTGGCATTTGGGATGTTCAAG-3’
KRAS	5’-GTAAAGGCGTGTTTGCTTAAACT-3’	5’-ACCAAGTCACCTGTTGTGTATC-3’
NRAS	5’-GATGTCCGTGGAAGTTGTAAGA-3’	5’-GGAGGCCAATAGTTCCTGTTTA-3’